The chelation of metal ions by dipeptides and related compounds

Many proteolytic enzymes are activated by metal ions[1,2,3] and hence a study of the interaction of the substrates with the appropriate metal ions is of value in the elucidation of the role of the metal ion activator in the catalytic process. Thus a knowledge of the sites of interaction in the metal-substrate complexes is necessary for any mechanistic interpretation and the stability constants of the complexes are required for any detailed kinetic analysis[4].

The interacton of metal ions with simple amino acids can be represented in the usual stepwise manner:

$$M^{++} + L^- \rightleftharpoons ML^+ \quad (1)$$
$$ML^+ + L^- \rightleftharpoons ML_2 \quad (2)$$

It has been shown[5,6] that the interaction of simple di- and tri-peptides (containing two ionizing groups) with cupric ions cannot be formulated in this manner because acid ionizations occur from both the 1:1 and 2:1 ligand-copper complexes, thus:

(a) ligand-copper 1:1 molar ratio

$$M^{++} + L^- \rightleftharpoons ML^+$$
$$ML^+ \rightleftharpoons ML + H^+ \quad (3)$$
$$ML \rightleftharpoons ML^- + H^+ \quad (4)$$

(b) ligand-copper 2:1 molar ratio

$$M^{++} + L^- \rightleftharpoons ML^+$$
$$ML^+ \rightleftharpoons ML + H^+$$
$$ML^+ + L^- \rightleftharpoons ML_2^- \quad (5)$$
$$ML_2^- \rightleftharpoons ML_2^{--} + H^+ \quad (6)$$

Two acid dissociations occur from the glycylglycine-copper 1:1 complex; the first (equation 3) has a pK value of the order of 4 and the second (equation 4) has a pK value of the order of 9.5. These have been assigned to the ionization of a peptide hydrogen atom and the uptake of an hydroxyl ion respectively[5].

In order to elucidate the nature of these interactions we have measured the stability constants of the complexes of a number of dipeptides and related compounds with the ions Cu^{++}, Co^{++} and Mn^{++}. Some of the results obtained are given in Table I; log K_1 and log K_2 refer to the equilibria shown in equations 1 and 2, and pK_c and pK_c' refer to the acid dissociations shown in equations 3 and 4. For the cationic acids the charge species in equations 1, 2, 3 and 4 are different but the equilibria involved are analogous. No acid ionizations were observed from the cobaltous and manganous complexes of any of the ligands. As shown in Table I acid dissociations from cupric complexes occur when the ligand has a peptide or amide hydrogen atom. Although no values are quoted for glycylglycine ethyl ester and glycinamide, acid dissociations nevertheless occur but quantitative data could not be obtained owing to hydrolysis of the ligand and precipitation in the solutions. If the peptide hydrogen atom is replaced by an alkyl group, as in glycylsarcosine and glycylproline, no acid dissociations occur from the cupric complexes. This is suggestive evidence that the peptide (or amide) hydrogen atom is the source of the acid ionization represented by equation 3; hence considerable interaction must occur between the cupric ion and the peptide nitrogen or oxygen atom. Since co-ordination at the peptide nitrogen will inhibit resonance in the peptide bond, the peptide oxygen is the most likely site of interaction.

TABLE I

THE STABILITY AND ACID DISSOCIATION CONSTANTS OF SOME DIPEPTIDES AND RELATED COMPOUNDS AT 25° C

	H^+	Cu^{++}			Co^{++}		Mn^{++}
	pK_a	log K_1	pK_c	pK_c'	log K_1	log K_2	log K_1
Glycylglycine	8.23	5.82	4.25	9.62	3.23	2.56	2.19
Sarcosylglycine	8.63	5.30	3.85	9.46	2.93	2.37	(0.4)
N-Dimethyl glycylglycine	8.03	4.75	3.85	9.19	2.08	2.16	—
Glycylglycine ethyl ester	7.79	4.66	—	—	2.22	1.84	—
Glycinamide	7.99	5.16	—	—	2.71	2.24	1.3–1.8
Glycylsarcosine	8.77	6.50	—	—	3.91	3.50	2.29
Glycylproline	8.66	6.66	—	—	3.90	2.95	2.29

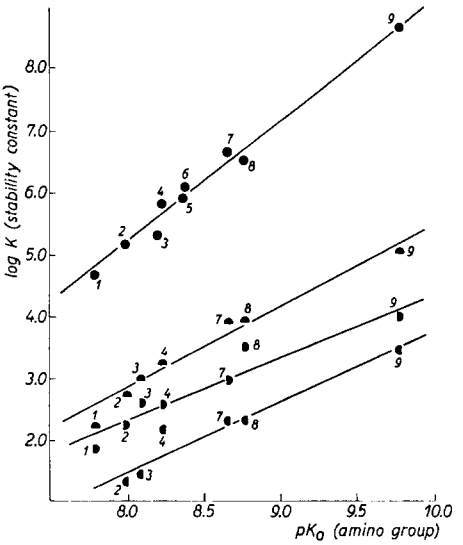
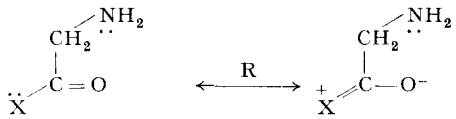


Fig. 1. Correlation of stability constants with pK of amino group. ● log K_1, Cu^{++}; ◕ log K_1, Co^{++}; ◗ log K_2, Co^{++}; ◖ log K_1, Mn^{++}. 1, glycylglycine ethyl ester; 2, glycinamide; 3, diglycylglycine; 4, glycylglycine; 5, glycyltyrosine; 6, glycylleucine; 7, glycylproline; 8, glycylsarcosine; 9, glycine. The data for the copper complexes of diglycylglycine, glycyltyrosine and glycylleucine are due to DOBBIE AND KERMACK[5,6] and are for 20° C. The data of EVANS AND MONK[9] have been employed for the glycine complexes and the cobaltous and manganous complexes of diglycylglycine.

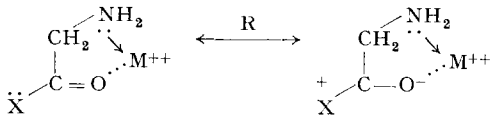
Further evidence for an interaction between the peptide oxygen atom and the metal ion is shown in Fig. 1, where the logarithms of the stability constants are plotted against the pK values of the amino groups of the ligands. It can be seen that for the compounds considered excellent straight line correlations are obtained; in these circumstances a reasonable assumption is that the ligands combine with any one of the metal ions in the same manner and through the same groups and, if ring formation occurs, the ring size is the same for all the ligands. The structural feature common to all these chelating agents is:



where X is:

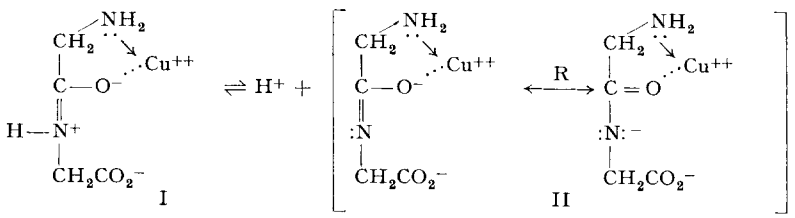
glycine	$-O^-$
glycinamide	$-NH_2$
glycylglycine	$-NH\cdot CH_2\cdot CO_2^-$
glycylsarcosine	$-N(CH_3)\cdot CH_2\cdot CO_2^-$
glycylglycine ethyl ester	$-NH\cdot CH_2\cdot CO\cdot OC_2H_5$
diglycylglycine	$-NH\cdot CH_2\cdot CO\cdot NH\cdot CH_2\cdot CO_2^-$

Hence the structure of the 1:1 ligand-metal complexes may be represented:



The N-metal bond is pictured as a co-ordinate link and the O-metal interaction as essentially electrostatic in accordance with the findings for glycine-metal chelates[7]. Data for sarcosylglycine and N-dimethyl glycylglycine are not included in Fig. 1 since additional steric factors are involved in the chelation of these ligands.

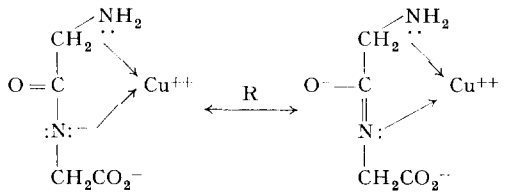
For the copper complexes of ligands other than glycylsarcosine and glycylproline ionization of the peptide hydrogen atom occurs subsequently to the initial formation of the complexes. A possible representation of this process is:



I II

glycylglycine:copper 1:1 complex

It is probable that complex II undergoes rearrangement to produce the structure:



This rearrangement, which might well occur simultaneously with the displacement of the peptide hydrogen, would be consistent with the marked change in absorption spectrum on ionisation of the complex[5].

These results have considerable bearing on the mode of action of the metal peptidases and lend some support to the theory of KLOTZ AND MING[8]. It has been suggested (see for example ref. [2]) that the role of the cobaltous ion activator in the activity of glycylglycine dipeptidase is to mediate the binding of the substrate to the enzyme. The failure of the enzyme to hydrolyse glycylsarcosine has been ascribed to the reluctance of this substance to form complexes with cobaltous ions. The results in Table I show this explanation to be false: glycylsarcosine is, in fact, a more powerful complexing agent than glycylglycine.

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Department of Biochemistry, University College London (England) S. P. DATTA
B. R. RABIN

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Zur Konstitution des Proteins des Tabakmosaikvirus

In früheren Arbeiten war festgestellt worden, dass die Aminoendgruppen im Protein des Tabakmosaikvirus (TMV) erst dann nachweisbar werden, wenn man das Virus mit schwachen Säuren (5%ige Trichloressigsäure) vorbehandelt. Nach der Säurebehandlung tritt Prolin als einzige N-terminale Gruppe in Ausbeuten von 1 Mol je 17,000 g Protein auf[1,2,3]. In der Peptidkette folgen auf das Prolin, Isoleucin und Glutaminsäure[4].

Wir versuchten die Endgruppe Prolin nach einem weiteren Verfahren freizulegen und ferner die Art der Blockierung festzustellen. Es ist bekannt, dass Amide des γ-Carboxyls der Glutaminsäure oder des β-Carboxyls der Asparaginsäure durch Säuren sehr viel leichter gespalten werden als α-Peptidbindungen. Wir untersuchten daher, ob die Blockierung auf einer derartigen γ- bzw. β-Peptidbindung der Seitenkette beruht.

WILLIAMS[5,6] konnte zeigen, dass durch Einwirkung von Hydroxylamin unter Transpeptidierung eine Spaltung der γ-Glutamylbindung unter gleichzeitiger Bildung der entsprechenden Hydroxamsäuren stattfindet. Diese Reaktion wurde näher untersucht und in Modellversuchen festgestellt, dass bereits unter schonenden Bedingungen durch Hydroxylamin Glutamin und Asparagin in die entsprechenden Hydroxamsäuren übergeführt werden. Hingegen reagieren α-Peptidbindungen unter den gegebenen Bedingungen nicht.

TMV wurde mit 3*N* Hydroxylaminlösung bei pH 6.5–7.5 24 Std. bei 60° im Brutschrank oder bei pH 7.0 1 Std. im Wasserbad bei 100° behandelt. In letzterem Fall wurde, um eine Ausflockung des Proteins zu verhindern, Dimethylharnstoff in einer Menge von ca. 50% hinzugegeben. In der Tat liess sich hiernach Prolin als Aminoendgruppe in einer Ausbeute von 50–80% der